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Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review)

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Abstract

Cholesterol oxidation products, termed oxysterols, are increasingly considered of potential interest in the pathogenesis of atherosclerotic lesions. Of dietary or endogenous origin, oxysterols may occur in significant amounts in low density lipoprotein (LDL) particles, especially in hypercholesterolemic subjects. They likely contribute to the uptake of modified LDL by scavenger receptors and some of them finally accumulate in the subintimal space of major arteries; here cholesterol oxides may favor the perpetuation of a chronic inflammatory state, through their ability to trigger irreversible damage of vascular cells with consequent activation of phagocytes. Furthermore, practically all oxysterols of major pathophysiologic interest have been shown to markedly up-regulate expression and synthesis of adhesion molecules, inflammatory cytokines and chemokines. Cholesterol oxidation thus appears to be an important biochemical pathway through which it exerts toxic, inflammatory and finally atherogenic effects.

Keywords: Oxysterols; Vascular cells; Apoptosis; Inflammatory cytokines; Atherosclerosis

1. Introduction

The association between plasma cholesterol and atherosclerosis has been a topic of intense research for many decades [1-5] and is still a major field of investigation. A considerable number of studies both in animals [6-9] and humans [10,11] have proved that prolonged high plasma cholesterol levels increase the risk of developing atherosclerosis. However, while hypercholesterolemia is now a well recognized primary risk factor of coronary heart disease, the mechanisms by which cholesterol contributes to the initiation, and in particular to the progression, of atherosclerotic lesions are still the subject of debate. The cholesterol molecule is not reactive *per se* as has been shown by feeding [12], venous infusion [13,14], and in vitro studies [15,16]. It must undergo structural changes to acquire sufficient reactivity. In this connection, several naturally occurring oxidation products of cholesterol, termed oxysterols, are of interest as possible reactive mediators of structural and functional changes of the vascular districts that are affected by the atherosclerotic process [17-26].

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2. Cholesterol oxidation products: oxysterols

Oxysterols are 27-carbon products of cholesterol oxidation; those of major biological interest are reported in Fig. 1. Introduction of an oxygen function increases the rate at which cholesterol is degraded to more polar compounds. Oxysterols, in particular those with additional oxygen functions in the steroid side chain, can easily be transported out of cells, and thus facilitate elimination of cholesterol from extrahepatic sources [27]. In addition to being intermediates in cholesterol degradation, oxysterols are a large class of potent regulatory molecules which have been shown to possess many important and diverse biochemical activities; however, their role in physiology is still controversial [19-24,26,28].

To improve the understanding of the biological significance of oxysterols, their physico-chemical features have been studied. Despite the presence of at least one oxygenated group in addition to the C3 beta-hydroxyl, oxysterols fit perfectly into the lipid bilayer of biological membranes exerting a condensing effect similar to that of cholesterol [23]. Lipid-protein interactions are probably crucial for the expression of the biochemical effects of oxysterols [21]; the most notable oxysterol activities deal with the regulation of key proteins of cholesterol homeostasis [29-32]. They may

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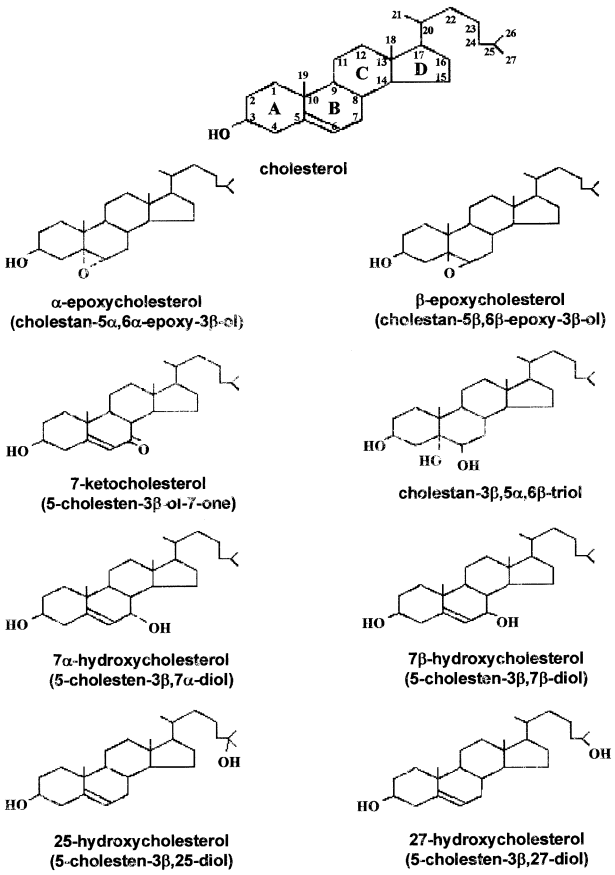

Fig. 1. Chemical structures of principal oxysterols of biological interest.

also serve as substrates for steroid hormone synthesis [33], and are intermediates in the transfer of sterol from the periphery to the liver [25]. Members of this class regulate the expression of genes that participate in both sterol and fat metabolism [34], and serve as substrates for the synthesis of bile acids [35-38].

Various oxysterols have been detected in appreciable quantities in human tissues and fluids. The concentration of oxysterols in biological fluids is determined by their rate of formation and metabolism, and in general the rate of metabolism of these compounds is considerably higher than that of cholesterol. Elevated levels of oxysterols have been found in human plasma and LDL subfractions, especially in LDL isolated from hypercholesterolemic subjects [24,39-41]; oxysterols are present in human macrophages/foam cells and atherosclerotic plaque and are suggested to play an active role in plaque development [42,43]. Indeed, oxidized cholesterol products have been shown to have greater atherogenicity than native cholesterol, as evidenced by arterial damage and development of arterial lesions [13,14,18,44].

3. Origin of in vivo detectable oxysterols

Oxysterols present in vivo may be either of exogenous origin, i.e. derived from the diet, or generated endogenously, as summarized in Table 1. Endogenous formation mainly occurs through autoxidation (non-enzymatic oxidation) of cholesterol [19,20], with some contribution also from enzymatic-driven convertion. Of note, some oxysterols of endogenous origin are exclusively produced via enzymatic reactions [45].

Table 1
Origin of plasma and tissue oxysterols

EXOGENOUS SOURCE
DIET:
• Already present in foods (meat, egg, milk, ...)
• Formed by autoxidation of foodstuff (induced by heat, light exposure, refrigeration)
ENDOGENOUS SOURCES
ENZYMATIC PATHWAYS:
• 7α-Hydroxylase
• 27-Hydroxylase
• 7-Ketone dehydrogenase
• 5α,6α-Epoxidase
NON-ENZYMATIC PATHWAYS:
• Attack by reactive oxygen species
• Attack by peroxyl and alkoxyl radicals from lipid peroxidation
• Leukocyte/H_2O_2/HOCl system

3.1 Dietary sources of oxysterols

Among foods and food products rich in cholesterol are eggs and dried egg powders (extensively used in a large number of commercial products) [46-48], milk powders (extensively used in infant formulas and other commercial foods), cheese and dairy products [49-51], red meat (beef, pork, veal) [52], ham, brain, liver, kidney, dried or stored fish (cod, anchovy, herring) [53,54].

All foods that contain cholesterol are also susceptible to oxidation [55], especially those which are dehydrated, subjected to radiation or high temperatures, or cooked in the presence of oxygen. Under all these conditions the foods are exposed to reactive oxygen species, such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$) and ozone (O_3). Not only processing but also storage for lengthy periods not under vacuum markedly increase the generation of oxysterols, essentially for the same reason.

The most commonly detected oxysterols in processed foods are 7-oxygenated sterols (7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol) and 5α,6α-oxygenated sterols (5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, cholestan-3β,5α,6β-triol); 25-hydroxycholesterol, 19-hydroxycholesterol, 20α-hydroxycholesterol, 3β-hydroxy-5α-cholestan-6-one and 3β,5α-dihydroxycholestan-6-one are present in much lower amounts [56-59].

3.2. Absorption of dietary oxysterols

Studies in humans [60,61] and animals [62-65] have demonstrated that dietary oxysterols are absorbed mainly in

the upper intestinal tract and then transported in the plasma within chylomicrons [60,63,64]. Circulating chylomicrons undergo conversion to cholesterol-rich chylomicron remnants by the action of endothelial lipoprotein lipase [66]. After lipolysis, these remnants are rapidly cleared by the liver [66-68].

Considerable discrepancy still exists among the few available reports in regards to the absorption rate of oxysterols in comparison to that of unoxidized cholesterol; it has been reported to be higher, lower or similar to that of the parent compound [13,41,62,64].

3.3. Oxysterols formed endogenously by non-enzymatic or enzymatic oxidation

Oxysterols may be generated within tissues through non-enzymatic oxidation of cholesterol, in general mediated by reactive pro-oxidant species [39,69] or via enzymatic catalysis [45].

In addition to the reactive oxygen species involved in the autoxidation of exogenous cholesterol, in animal tissues autoxidation may also be supported by peroxyl ($ROO^{\cdot}$) or alkoxyl (RO) radicals derived from lipid peroxidation [19, 20]. Other reactive species may derive from activation within macrophages and neutrophils (of the myeloperoxidase-driven $HOCl/H_2O_2$ system) [22], that occurring during atherosclerotic degeneration of the artery wall.

It appears credible that non-enzymatic routes play some role in the production of oxysterols, since plasma or tissue levels decrease in animals fed antioxidants such as vitamin E, butylated hydroxytoluene or probucol [69-72]. However, further investigation is needed to properly evaluate the full impact of autoxidation in the endogenous production of cholesterol oxides.

Some of the most abundant oxysterols found in vivo are enzymatic products of cholesterol catabolism. In mammals, the main enzymatic pathway involving cholesterol is its mono-hydroxylation in the synthesis of bile acids and steroid hormones. Two key enzymes of bile acid biosynthesis are 27-hydroxylase and 7α-hydroxylase [73,74]. The enzyme sterol 27-hydroxylase plays an important role in the hepatic degradation of the side-chain of cholesterol, during the formation of bile acids [45,75]. This enzyme is present not only in hepatocytes but also in several other cell types, including leukocytes and endothelial cells [76-78] where it appears involved in the elimination of cholesterol [45].

The synthesis of essential 7α-hydroxylated bile acids in the liver is mediated by two pathways that involve distinct 7α-hydroxylases [79]. 7α-hydroxylase activity has also been detected in kidney, heart, and lung microsomes [22]. Moreover, both 7α-hydroxycholesterol and 7β-hydroxycholesterol products appear to be generated by these enzymes, with the additional possible involvement of enzymatic inversion between the two epimers [22].

Several enzymes appear to be involved in oxysterol production: 7-ketone dehydrogenase generates 7α- or 7β-hydroxycholesterol and cholesterol-5α,6α-epoxidase produces 5α,6α-epoxycholesterol [19,22]. Finally, there is solid evidence of enzymatic conversion of cholesterol to 7-ketocholesterol and of 7-ketocholesterol to 7β-hydroxycholesterol [80-83] at least in the liver, which, incidentally, is where LDL is produced.

The actual contribution of enzymatic and non-enzymatic pathways to the total amount of oxysterols found in vivo remains unclear, but the prevaling opinion is that both pathways may have a considerable biological impact.

3.4. Metabolism of oxysterol

Once present in the organism, a specific oxysterol may follow two fates: it may be rapidly metabolized and excreted with the feces, or it may be distributed to the tissues where it may be metabolized. It has been demonstrated that oxysterols are rapidly cleared from the plasma by tissues and organs where they are submitted to partial metabolism followed by recycling to the liver via lipoproteins [65]. In the liver they are rapidly oxidized to more polar steroids and bile acids by the above enzymatic pathways, and are then excreted into the intestine as water-soluble products [84,85].

Alternatively, oxysterols may be esterified to long-chain fatty acyl esters either by intracellular acylCoA: cholesterol acyltransferase (ACAT) or by extracellular lecithin:cholesterol acyltransferase (LCAT). Because of their supposed cytotoxicity, esterification may be a means of protective metabolism [19]. As fatty acyl esters, oxysterols may accumulate in the tissues or undergo endogenous metabolism and subsequent transport for utilization or excretion.

An increasing body of evidence indicates that macrophages may accumulate both non-esterified and esterified oxysterols, an event associated with the atherosclerotic process [23,86-91].

Many hypotheses have been put forth to explain the intracellular accumulation of oxysterols, which have been shown to impair cholesterol homeostasis [20,24] through several mechanisms. The same mechanisms could be applied to explain their own homeostasis [86]; they include suppression of hormone-sensitive lipase, an enzyme necessary for the neutral cholesteryl ester hydrolase activity [92], activation of ACAT with subsequent increased formation of ester lipid droplets [20,23,86], derangement of plasma membrane structure and properties [86], damage to lysosomes with inactivation of cholesteryl ester hydrolase [93]. Moreover, it has been proved that extracellular acceptors of cholesterol, such as apoA-I, are less able to bind oxysterols, whose excretion is therefore less efficient [86,94].

Finally, since 27-hydroxylase appears to be one way in which cells avoid excessive cholesterol and oxysterol loading [84,95], an accumulation of these compounds could be explained by an alteration of 27-hydroxylase activity [96].

4. Concentration of oxysterols in plasma LDL

Oxysterols may account for a sizeable fraction of the oxidized lipids in the circulating LDL, depending on their specific intake with the diet and on the overall balance between prooxidant and antioxidant compounds in the blood stream. The previous section analyzed the chief products of cholesterol oxidation and their nutritional sources. Chronic liver diseases and diabetes mellitus may persistently perturb the redox balance in the hematic district. While liver diseases are not considered primary risk factors for atherosclerosis, most such diseases are associated with local sustained imbalance of the ratio between oxidative and reductive biochemical reactions towards oxidation, a condition termed "oxidative stress". Not only chronic alcohol intoxication but also iron and copper overload, cholestatic syndrome and chronic active hepatitis are all characterized by oxidative stress [97]. A major consequence of an increased steady-state level of reactive oxygen species (ROS) in hepatocytes is undoubtedly enhanced lipid peroxidation. Oxidative breakdown of membrane polyunsaturated fatty acids is followed by the generation of relatively reactive and diffusable end products, in particular those provided with an aldehyde functional group [98]. Among aldehyde end products of lipid peroxidation, 4-hydroxynonenal (HNE) is now recognized as having potential interest in pathobiology, due to its multiple biochemical effects at concentrations actually detectable in a large number of human diseases [99,100] and related lesions including the atherosclerotic plaque [101]. HNE is deemed to play a significant role in the process of "modification" of LDL, in addition to other aldehydes, such acetaldehyde and glucose itself [98-102]. In diabetes mellitus increased plasma levels of HNE are also constantly detected both in the free form and covalently bound to proteins and lipids [103].

It would be interesting to know if lipoprotein cholesterol may also undergo oxidative modifications under conditions of hepatic oxidative stress, in particular in alcoholics. At present, no relevant studies are available, although LDL is known to be avidly taken up from hypercholesterolemic blood by activated Kupffer cells [104]. The only report available is a rather old one that describes a marked decrease of rat liver 7α-hydroxylase activity after chronic ethanol intoxication [105]. If this evidence were confirmed, it may suggest that sustained impairment of cholesterol degradation in the liver allows the accumulation of both the sterol and its more toxic metabolites.

Whether or not hepatic metabolism affects the normal cholesterol oxidation rate, oxysterols in human plasma or serum may vary from about 1 μM (0.05% of total cholesterol) in healthy subjects to 20-30 μM (0.5-0.75% of total cholesterol) in diseased individuals [26]. Much higher concentrations of plasma oxysterols have also been reported; in this respect, though, we fully agree that, because of the easy generation of oxidized cholesterol species during LDL isolation and analysis, only those studies that include rigorous internal controls should be considered credible [26]. As reported below, oxysterols display a wide spectrum of activities of pathobiological interest, even in the low micromolar range. Of note, most (70-80%) of the oxysterols of biological interest, are consistently recovered in the LDL, but significant fractions are also detectable in high density lipoprotein (HDL) and very low density lipoprotein (VLDL) [40,106]. In human plasma LDL, lipid oxidation products tend to concentrate in an electronegatively charged subfraction, termed LDL$^-$, which can be separated from bulk LDL by ion exchange high pressure liquid chromatography [107]. LDL$^-$ may account from 0.5 to 5-10% of total LDL and is enriched in cholesterol oxides, α-epoxycholesterol, cholestane-3β,5α,6β-triol, 7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol being among the most abundant [108].

5. Oxysterols and receptors for LDL

Since Brown and Goldstein have proposed many years ago the possible contribution of oxysterols in receptor-mediated LDL uptake [109], other scientists have shown that specific oxysterols may suppress LDL binding activity in cultivated human fibroblasts and hepatocytes [26]. The marked decrease of LDL uptake and processing was consistently observed after sustained incubation of sensitive cells to ^{125}I-LDL, i.e., from 6 to 48 hr, while it was not evident at shorter incubation times, thus suggesting a transcriptional effect of oxysterols on the LDL receptor gene. Consistent evidence has been provided of a marked down-regulation of expression and synthesis of LDL receptors by specific oxysterols. In this regard, it should be pointed out that 25-hydroxycholesterol, i.e. the molecule most often tested in those studies, is actually 5-10 times less abundant in oxidized LDL than other oxysterols, such as 7α-hydroxyl- or 7β-hydroxycholesterol [108]. The potential effect of oxysterols on the expression of LDL receptors may be exerted once the lipoprotein is internalized in the vascular cells, and an action of this kind might indirectly amplify the uptake of modified and oxidized LDL by scavenger receptors. Moreover, very recent data clearly indicate how, in the most electronegative subfraction (LDL$^-$) of plasma LDL, apoB-100 undergoes a profound conformational change, likely due to oxidative degradation; the consequence is the sinking of the apolipoprotein into the lipid environment, an event favored by the decreased packing of the surface lipids, which probably accounts for the reduced affinity of this LDL fraction for LDL receptors [110].

The expression of the gene coding for LDL receptor is actually regulated by defined transcription factors called sterol regulatory element binding proteins (SREBPs). These proteins also favor transcriptional activation of genes encoding enzymes of cholesterol and fatty acid synthesis. Maturation of SREBP consists of proteolytic cleavage of the original molecule, which is dependent upon the SREBP

cleavage activation protein (SCAP). After proteolytic remodeling, SREBP is then able to translocate in the nucleus and act as transcription factor [24]. Several oxysterols have been shown able to bind to SREBPs and depress both their maturation and nuclear localization [26,111]. Such inhibition, that may results in a down-regulation of cholesterol synthesis, is recognized as a primary mechanism by which cells counteract an excess of cholesterol intake. The steps of this response are: cholesterol intake, its partial conversion into oxysterols, down-regulation of SREBP proteolytic activation, reduced expression of LDL-receptor and reduced cholesterol synthesis [29]. In conclusion, oxysterols down-regulate the expression of LDL receptors on vascular cells. As a consequence, LDL cholesterol is preferably taken up by scavenger receptors expressed on macrophages, by this way favoring foam cell formation.

6. Oxysterol toxicity towards vascular cells

Lipid oxidation products including oxysterols have consistently been found not only in circulating LDL but also in the characteristic lesions of atherosclerosis, thus in the arterial wall. Many data are now available on the toxicology of the oxysterols of pathobiological interest. Indeed, the focal toxicity of cholesterol oxidation products, transported to the subintimal space by LDL and accumulating there, may play a role in the evolution of the necrotic core region of atherosclerotic plaques. This region, characterized by intense lipid deposition and cell necrosis, lies for long periods under the relatively normal endothelial layer, but with time it expands, eventually leading to irreversible damage of vascular endothelial cells, platelet adhesion and aggregation, and thrombotic events.

A brief review follows of the literature appearing over the last decade on the antiproliferative, cytotoxic and proinflammatory effects of oxygenated derivatives of cholesterol; however, it should be borne in mind that practically all toxicologic studies have been carried out on the major oxysterols taken individually, while in biology they always occur in mixtures. In addition, unoxidized cholesterol had almost never been used for comparative analysis.

6.1. Apoptosis induced by oxysterols

The induction of apoptosis in cells of the arterial wall is undoubtedly a primary process in the pathogenesis of atheroma. The increasing number of in vitro demonstrations that several oxysterols mediate programmed cell death in a biologically compatible concentration range is relevant to the recognized proapototic effect of oxidized LDL.

Among the first reports on this matter, Christ et al. showed that the T-cell response to different stimuli was strongly inhibited by treatment with 25-hydroxycholesterol, and that an apoptotic program was on the contrary triggered [112]. Aupeix et al. showed similar apoptotic effect occurring shortly after treatment of human monocytic cell lines with either 25-hydroxycholesterol or 7-ketocholesterol [113]. Interestingly, when cells were simultaneously incubated with equimolar amounts of the two oxysterols, a significant quenching of apoptosis was observed [113]. Several other in vitro studies then reported that 25-hydroxycholesterol, 7-ketocholesterol and also 7β-hydroxycholesterol were able to induce apoptosis in human vascular cells, namely artery smooth muscle cells [114,115], endothelial cells [115,116], and monocyte-macrophages [116-118].

In the pathogenesis of atherosclerosis, the ability of oxysterols, once accumulated in the subintimal space, to progressively injure the endothelial barrier and to damage the main structural component of the vascular wall, i.e., smooth muscle cells, is highly significant. Further, the oxysterol-induced death of macrophages may be a valid explanation of why cholesterol and other lipids accumulate in the artery walls, without being removed by phagocytosis.

Scattered information relating to the pathomechanics underlying the proapoptotic action of naturally occurring oxysterols has appeared over recent years. A down-regulation of the antiapoptotic protein bcl-2 has been demonstrated in different cell models and validated by the protective effect of bcl-2 overexpression [114,117,119]. These studies indicate the involvement of the mitochondrial pathway of apoptosis. Further, an excessive production of ROS with consequent redox imbalance was shown to be associated with internucleosomal DNA fragmentation provoked by specific oxysterols [120].

Only recently, more comprehensive data on the pathogenesis of cell death by this class of compounds has appeared. Treating human aortic smooth muscle cells with 7-ketocholesterol, Ares et al. [121] showed that the mitogen-activated protein kinase (MAPK) pathway is involved, after triggering by perturbation of Ca^{2+} intracellular homeostasis. Extracellular signal-regulated kinase 1 (ERK 1) and 2 (ERK 2) but not Jun-N-terminal kinases (JNK) were activated immediately after cell challenge with the sterol [121]. Again using vascular smooth muscle cells, this time incubated with 25-hydroxycholesterol or 7β-hydroxycholesterol, Lee and Chau [122] demonstrated that an overexpression of the programmed death mediators Fas and Fas ligand was at least partly involved. Of note, α-tocopherol and deferoxamine were able to quench such overexpression, indirectly suggesting the involvement of ROS in the initiation step [122]. Loss of mitochondrial transmembrane potential, cytosolic release of cytochrome c and activation of caspase 9 have been demonstrated in human promonocytic cells challenged with 7α- or 7β-hydroxycholesterol or 7-ketocholesterol [123]. As is clear in the review by Panini and Sinensky, in the low micromolar range oxysterols induce death of various vascular cells through activation of both intrinsic and extrinsic apoptotic pathways [124].

6.2. Do oxysterols exert necrogenic effect on vascular cells?

While the classic dogma that apoptosis never triggers inflammatory reaction is now becoming arguable [125], a necrogenic effect of oxysterols on vascular cells would certainly be a valid proinflammatory stimulus. Thus, while examining the proatherogenic effect of oxysterols, it is important to clarify whether, under specific conditions, these compounds exert necrogenic activity. Lizard et al. [115] recently characterized the mode of vascular cell death following acute intoxication with 7-ketocholesterol or 7β-hydroxycholesterol, up to a final concentration of 12-200 μM. Within this concentration range, the two sterols consistently induced apoptosis in both human endothelial cells and smooth muscle cells, while fibroblasts isolated from human umbilical cord veins were irreversibly damaged through necrotic events [115]. In monocytic cells, we demonstrated that 7-ketocholesterol induced apoptosis of cultivated macrophages at 10-30 μM, while at higher concentrations apoptotic death could not clearly be distinguished from necrotic death (Leonarduzzi, unpublished observations).

Our strong impression from these and other reports is that, apart from the significantly different susceptibility of the various vascular cells to oxysterol-mediated toxicity, apoptosis is the mode of cell death that prevails under this type of challenge at low micromolar concentration. Necrosis is more pronounced in the presence of oxysterols in medium-high micromolar concentrations, which are probably only reached in the core of the atherosclerotic lesions. In this connection, several years ago, Guyton et al. [126] developed a valid model to study the atheromasic core region, which they found mainly to consist of a layer of collagen embedded with crystals of cholestane-3β,5α,6β-triol, or 25-hydroxycholesterol or unoxidized cholesterol, on which canine aortic smooth muscle cells were seeded. Oxysterols, but not cholesterol, were able to reduce cell plating efficiency and focal areas of necrosis.

7. Proinflammatory (proatherogenic) effect of oxysterols in the vasculature

It is generally accepted that vascular areas of atherosclerotic lesion progression, are in a state of persistent inflammation. As a consequence, any further inflammatory stimulus in the subintimal area automatically becomes a proatherogenic stimulus [127].

An increasing body of evidence supports a proinflammatory and proatherogenic role of oxidized LDL. Oxidized LDL has been demonstrated to up-regulate the expression of inflammatory cytokines such as interleukin-1 (IL-1) and interleukin-8 (IL-8) in monocytic cells [128], tumor necrosis factor-α (TNF-α) in monocytic cells [129], and chemokines such as monocyte chemotactic protein-1 (MCP-1) in endothelial and smooth muscle cells [130].

However, it still remains to be clarified which components of oxidized LDL are actually responsible for all these effects. It seems quite likely that oxysterols may contribute to the proinflammatory effect of oxidized LDL. This is increasingly supported by evidence of modulation of proinflammatory molecules by cholesterol oxidation products, which indeed accumulate in human fibrotic plaques. In this regard, treatment of smooth muscle cells with 25-hydroxycholesterol has been reported to increase both mRNA levels and synthesis of basic fibroblast growth factor (bFGF), a cytokine with potent mitogenic and fibrogenic activities [131]. Moreover, the treatment of human umbilical vein endothelial cells (HUVECs) with 7-ketocholesterol, 7α-hydroxycholesterol and especially with 7β-hydroxycholesterol induced a 10-20 fold increase of IL-1β secretion [116]. The proinflammatory role of these three sterols appears to occur at different levels, including endothelial adhesion and extravasation of leukocytes. All three oxysterols induce the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial-selectin (E-selectin) in HUVECs [116]. Of note, these adhesion molecules have been found to be markedly overexpressed in hypercholesterolemic patients [132], thus it is reasonable to attribute this gene expression effect to oxysterols also in vivo.

Increasing evidence is accumulating of the gene expression effect of oxysterols on monocytes/macrophages, by far the most represented leukocytes in atheromatous lesions. A strong induction of IL-1β by 7-ketocholesterol was reported some years ago in the human promonocytic cell lines U937 and U4 [119]. More recently, using human monocytes and monocyte-derived macrophages, Liu et al. showed that marked up-regulation of the chemotactic IL-8 was sustained by specific oxysterols, including 25-hydroxycholesterol and 7β-hydroxycholesterol [133]. In addition, clear evidence has now been provided of increased expression and synthesis of transforming growth factor β1 (TGFβ1), the cytokine with by far has the strongest profibrogenic effect [16]. In this case, a mixture of oxysterols, in a percent concentration representative of that detectable in LDL, was added to J774 and U937 cells of the macrophage lineage.

The synthesis of another cytokine with strong proinflammatory effect, namely TNF-α, was found to be up-regulated by 22-hydroxycholesterol in human peripheral monocytes as well as in the human monocytic cell line THP-1 [134]. However, in the case of TNF-α, further evidence with different cells and with other oxysterols appears necessary, partly because such up-regulation was not observed with endothelial cells challenged with the oxysterols, which mainly accumulate in atherosclerotic lesions [116].

8. Concluding remarks

General agreement has been reached over the last few years on the potential toxicity of oxysterols circulating in

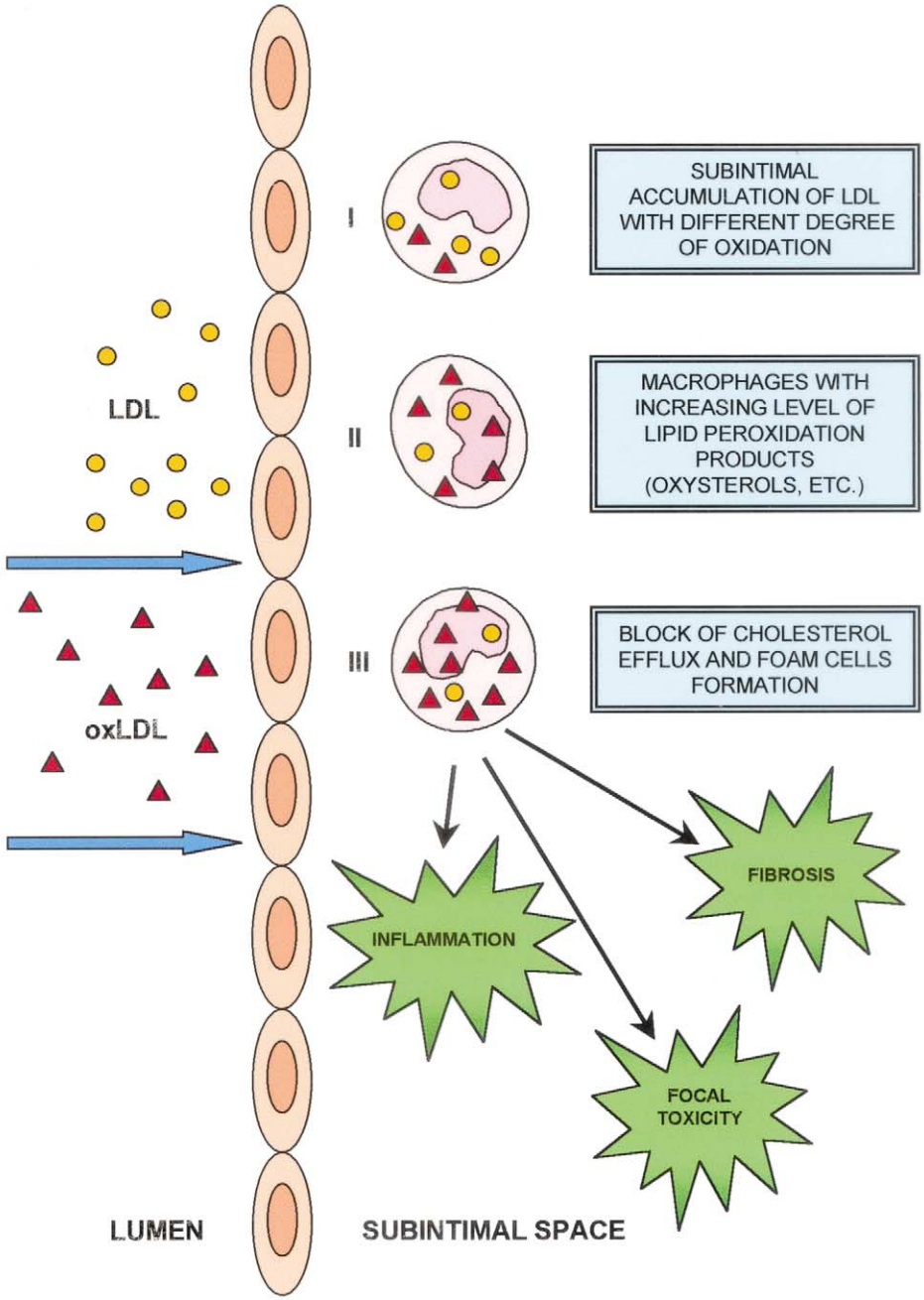

Fig. 2. Schematic diagram. In the subintimal space of major arteries, the phagocyte uptake of LDL particles, previously oxidized in the lumen or still undergoing oxidation with subsequent formation of lipid peroxidation products, such as oxysterols, modulates pathobiological events which are correlated to atherosclerotic process.

LDL and possibly accumulating in the subintimal space of major arteries. Apart from the actual oxysterol content in circulating lipoproteins, which is undoubtedly influenced by the dietary regimen, a mixture of cholesterol oxides can be found in the LDL, while only few of them appear to accumulate in atherosclerotic lesions. In other words, differential metabolism presumably occurs within the oxysterol class, thanks to the activity of some specific enzymes. Further, the deposition of oxysterols in the subintimal space of the artery is quantitatively influenced by oxidative events which probably occur there, mainly as a consequence of a sustained inflammatory state, a major role being played by activated phagocytes. Accumulated oxysterols are a valid and sustained inflammatory stimulus, through their ability to significantly enhance apoptosis, necrosis and cytokine production (Fig. 2). In this regard, it would be extremely interesting to extend the toxicological analysis to mixtures of oxysterols rather than single compounds. Very recently, equimolar amounts of 7-ketocholesterol and of a mixture of oxysterols, representative of that found in oxidized LDL,

were compared in means of their apoptotic effect on cultivated macrophages. At least in the concentration range 10 and 30 μM, only the oxysterol when used alone strongly induced apoptosis [16]. Such evidence is supported by the very few reports on the quenching effect of oxysterol toxicity by the simultaneous administration of equimolar concentrations of a second cholesterol oxide [113]. Thus, mixtures of oxysterols like those occurring in the LDL may prove to be less toxic towards vascular cells than single, purified oxysterols. This would certainly favor both the uptake and the subintimal localization of LDL particles rich in oxidized molecules, without triggering acute damage. At least under certain conditions, LDL could be compared with a Trojan horse [135]. Through the uptake of modified LDL, an increasing number of potentially toxic compounds are deposited behind the endothelial wall, where they may exert toxicity, once properly concentrated and further metabolized. Oxysterols appear suitable candidate molecules for this kind of process.

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